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ORIGINAL ARTICLE

The dual glucose-dependent insulinotropic peptide and glucagon-like peptide-1 receptor agonist, tirzepatide, improves lipoprotein biomarkers associated with insulin resistance and cardiovascular risk in patients with type 2 diabetes

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Abstract

Aim: To better understand the marked decrease in serum triglycerides observed with tirzepatide in patients with type 2 diabetes, additional lipoprotein-related biomarkers were measured post hoc in available samples from the same study.

Materials and Methods: Patients were randomized to receive once-weekly subcutaneous tirzepatide (1, 5, 10 or 15 mg), dulaglutide (1.5 mg) or placebo. Serum lipoprotein profile, apolipoprotein (apo) A-I, B and C-III and preheparin lipoprotein lipase (LPL) were measured at baseline and at 4, 12 and 26 weeks. Lipoprotein particle profile by nuclear magnetic resonance was assessed at baseline and 26 weeks. The lipoprotein insulin resistance (LPIR) score was calculated.

Results: At 26 weeks, tirzepatide dose-dependently decreased apoB and apoC-III levels, and increased serum preheparin LPL compared with placebo. Tirzepatide 10 and 15 mg decreased large triglyceride-rich lipoprotein particles (TRLP), small low-density lipoprotein particles (LDLP) and LPIR score compared with both placebo and dulaglutide. Treatment with dulaglutide also reduced apoB and apoC-III levels but had no effect on either serum LPL or large TRLP, small LDLP and LPIR score. The number of total LDLP was also decreased with tirzepatide 10 and 15 mg compared with placebo. A greater reduction in apoC-III with tirzepatide was observed in patients with high compared with normal baseline triglycerides. At 26 weeks, change in apoC-III, but not body weight, was the best predictor of changes in triglycerides with tirzepatide, explaining up to 22.9% of their variability.

Conclusions: Tirzepatide treatment dose-dependently decreased levels of apoC-III and apoB and the number of large TRLP and small LDLP, suggesting a net improvement in atherogenic lipoprotein profile.

KEYWORDS
incretin therapy, type 2 diabetes

1 | INTRODUCTION

Despite the current standard of care treatment, patients with type 2 diabetes (T2D) have a high residual risk of atherosclerotic cardiovascular events.[1] Most of these patients have atherogenic dyslipidaemia, which is characterized by high levels of fasting and postprandial triglycerides, low levels of high-density lipoprotein cholesterol (HDL-C) and high levels of small low-density lipoprotein particles (LDLP) .[2,3] The increase in large very-low-density lipoprotein (VLDL) particles is at the root of the atherogenic dyslipidaemia in T2D, which is partially secondary to insulin resistance.[4] While statins reduce low-density lipoprotein cholesterol (LDL-C) and the number of LDLP, their effect on triglyceride-rich lipoproteins and their remnants is minimal.[5] Apolipoprotein (apo) C-III is a key regulator of triglyceride metabolism.[6–8] It is well known that apoC-III is the inhibitor of lipoprotein lipase (LPL), the rate-limiting enzyme of the hydrolysis.[9,10] Recent data also suggest that apoC-III inhibition reduces plasma triglycerides by LPL-independent pathways by enhancing hepatic uptake of triglyceride-rich lipoprotein (TRL) remnants via low-density lipoprotein receptor and low density lipoprotein receptor-related protein 1 receptors in the liver.[8,11] Glucagon-like peptide-1 (GLP-1) receptor agonists have been shown to be effective in reducing major adverse cardiac events in T2D.[12–14] Numerous mechanisms beyond glucose lowering have been considered as possible contributors to their cardioprotective effects. These mechanisms include weight loss, reducing blood pressure, renal protection, and lowering chronic inflammation and liver fat, which are all known to be associated with cardiovascular risk.[15–17] GLP-1 receptor agonists have been shown to reduce lipoprotein and chylomicron production, as well as postprandial triglycerides, VLDL cholesterol and free fatty acids,[18–20] but the magnitude of these effects is not large.

The effect of chronic treatment of glucose-dependent insulinotropic peptide (GIP) receptor agonists on plasma lipids is not well known.[21] GIP receptor expression in adipose tissue suggests a role as a potential regulator of dietary lipid clearance in response to postprandial GIP secretion. Indeed, in preclinical in vivo and in vitro models, GIP increases adipocyte LPL expression.[22,23] Although the mechanisms are not fully understood, infusion of GIP and GIP receptor antagonists in humans suggests that GIP receptor activation increases adipose tissue blood flow and promotes adipose tissue lipid uptake.[24,25] Thus, GIP receptor agonism in adipocytes may be a key regulator of postprandial lipid clearance and potentially overall lipid homeostasis.

However, dual agonism of GIP and GLP-1 receptors appears to have a greater effect on lipid and lipoprotein metabolism in vivo in humans. We have recently shown that the effect of the dual GIP and GLP-1 receptor agonist, tirzepatide, on plasma triglyceride is much larger than that of the GLP-1 receptor agonist, dulaglutide.[26] At week 26, tirzepatide at different doses and dulaglutide 1.5 mg did not differ in terms of changes in the concentrations of total cholesterol, LDL-C or HDL-C.[26] In the current study, we report additional effects of tirzepatide on the levels of apolipoproteins and lipoprotein particle subclasses measured in stored samples from the same phase 2b study of tirzepatide in patients with T2D.

2 | MATERIALS AND METHODS

2.1 | Participants

A detailed description of participant eligibility, study design and efficacy and safety results has been published.[26] Briefly, this was a double-blind, placebo-controlled phase 2b clinical trial, where patients were randomized (1:1:1:1:1:1) to receive either once-weekly subcutaneous tirzepatide (1, 5, 10 or 15 mg), dulaglutide (1.5 mg) or placebo for 26 weeks. This trial (ClinicalTrials.gov identifier: NCT03131687) was conducted in accordance with the Declaration of Helsinki and the Council for International Organizations of Medical Sciences International Ethical Guidelines, the International Conference on Harmonization Good Clinical Practices Guideline, and all other applicable laws and regulations. All participants provided written consent prior to any procedure.

2.2 | Study procedures

Samples were collected in the fasting state. Triglycerides and LDL-C were measured on fresh serum samples using traditional enzymatic methods. Non-HDL-C was calculated as total cholesterol minus HDL-C. All other measurements of apolipoproteins and lipoprotein particle subclasses (triglyceride-rich lipoprotein particles [TRLP], LDLP and high-density lipoprotein particles [HDLP]) were performed on available frozen plasma EDTA or serum samples from the modified intent-to-treat population (mITT) population. Lipoprotein particle concentration and average lipoprotein particle size were measured by nuclear magnetic resonance (NMR) spectroscopy at baseline and 26 weeks (LabCorp, Burlington, NC, USA). The lipoprotein insulin resistance (LPIR) score, a weighted combination of six lipoprotein subclass measures (large TRLP, large HDLP, small LDLP and mean sizes of TRLP, LDLP and HDLP), was also calculated.[27] Preheparin serum LPL mass was measured by enzyme-linked immunosorbent assay (ALPCO, Salem, NH,

USA). ApoA-I, apoB (Roche, Indianapolis, IN, USA) and apoC-III (Kamiya, Seattle, WA, USA) were measured by immunoturbidimetry at baseline and at 4, 12 and 26 weeks.

2.3 | Statistical analyses

Patients from the mITT (without data after study drug discontinuation or rescue drug initiation) were included in this analysis. Baseline measurements of biomarkers were analysed using analysis of variance (ANOVA) while postbaseline measurements were analysed using mixed model with repeated measure. Biomarkers with skewed distribution were log-transformed at baseline prior to the analysis. A two-sided *P*-value of .05 was used as significance level. Biomarkers measured by NMR spectroscopy were analysed by analysis of covariance (ANCOVA) values and treatment group as covariates. Large and medium LDLP data contained many zeros, and therefore were analysed using the Tobit regression.[28]

The proportion of variability in triglyceride changes explained by potential predictors, like age, sex, baseline HbA1c, baseline body weight, baseline triglycerides, baseline apoC-III, baseline homeostatic model assessment of insulin resistance (HOMA-IR), HbA1c change, body weight change, HOMA-IR change and apoC-III change, was evaluated. A stepwise variable selection based on the Akaike information criterion was performed on pooled data of 10 and 15 mg tirzepatide doses in order to select the best fit model among all possibilities. ANOVA with type-III sums of square was conducted on the output of multiple linear regression analysis based on the final selected model to compute the explained variability of each selected variable. The selected model and the variability explained by each of its variables are summarized in Table S5. Statistical analyses were performed using R 3.6.0, SAS 9.4 software (Cary, NC, USA) and GraphPad Prism 8 (San Diego, CA, USA).

3 | RESULTS

3.1 | Baseline characteristics

Baseline demographics, clinical characteristics and lipid profile were similar across all treatment groups (Tables **S1** and S2).[26]

3.2 | Lipid biomarker profile

The lipid profile at baseline and change from baseline at 26 weeks is presented in Table S2. Tirzepatide dose-dependently decreased triglycerides over time compared with placebo (Figure 1A). At 26 weeks, tirzepatide 5 mg, 10 mg and 15 mg and dulaglutide decreased triglyceride levels by 28.8% (−39.0%, −16.7%; *P* < .001), 37.7% (−46.8%, −26.9%; *P* < .001), 41.4% (−50.5%, −30.7%; *P* < .001) and 18.8% (−30.6%, −5.0%; *P* = .010)

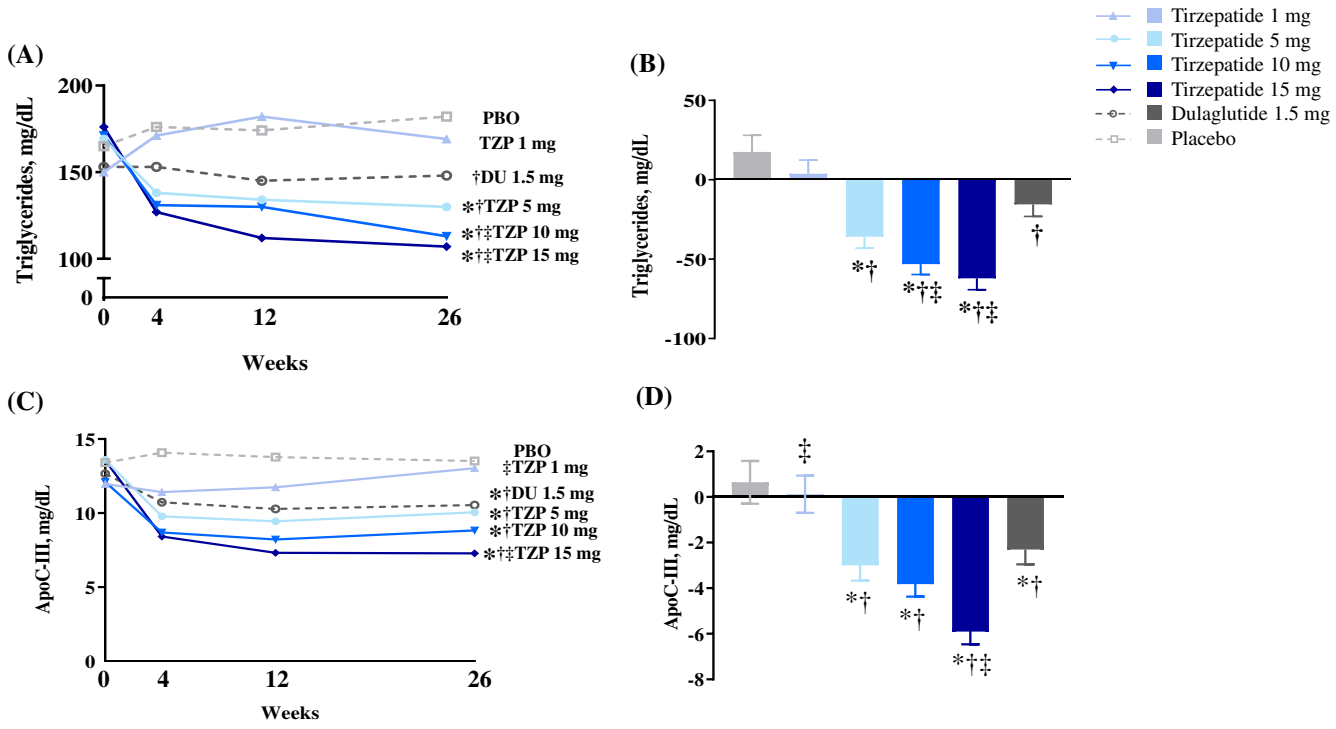


FIGURE 1 Triglycerides and ApoC-III over time and change from baseline at 26 weeks. A, Change from baseline over time in triglycerides. B, Change from baseline at 26 weeks in triglycerides. C, Change from baseline over time in ApoC-III. D, Change from baseline at 26 weeks in ApoC-III. Data are presented as LSM actual values over time and LSM (SE) change from baseline at 26 weeks from the mITT population (placebo, n = 51; tirzepatide 1 mg, n = 52; tirzepatide 5 mg, n = 55; tirzepatide 10 mg, n = 51; tirzepatide 15 mg, n = 53; dulaglutide 1.5 mg, n = 54). Triglycerides and apoC-III data are presented as antilog. **P* < .05 vs. baseline, †*P* < .05 vs. placebo, ‡*P* < .05 vs. dulaglutide. ApoC-III, apolipoprotein C-III; DU, dulaglutide; PBO, placebo; TZP, tirzepatide

(least squares mean [LSM] [95% CI]), respectively, compared with placebo (Figure 1B). Tirzepatide 10 mg and 15 mg also decreased triglyceride levels by 23.3% (−34.3%, −10.5%; $P < .001$) and 27.9% (−38.9%, −15.0%; $P < .001$), respectively, compared with dulaglutide.

Tirzepatide dose-dependently also decreased apoC-III levels over time compared with placebo (Figure 1C). At 26 weeks, tirzepatide 5 mg, 10 mg and 15 mg and dulaglutide decreased apoC-III levels by 25.6% (−38.0%, −10.8%; $P = .002$), 34.8% (−45.8%, −21.4%; $P < .001$), 46.2% (−55.7%, −34.6%; $P < .001$) and 22.0% (−34.9%, −6.5%; $P = .007$) (LSM [95% CI]), respectively, compared with placebo (Figure 1D).

As with triglyceride and apoC-III levels, tirzepatide dose-dependently decreased apoB levels over time compared with placebo (Figure 2A). At 26 weeks, tirzepatide 5 mg, 10 mg and 15 mg and dulaglutide decreased apoB levels by 11.0% (−18.2%, −3.3%; $P = .006$), 14.8% (−21.8%, −7.2%; $P < .001$), 17.4% (−24.5%, −9.5%; $P < .001$) and 14.1% (−21.0%, −6.7%; $P < .001$) (LSM [95% CI]), respectively, compared with placebo (Figure 2B). The magnitude of reduction in apoB levels was not significantly different between tirzepatide and dulaglutide. Increases from baseline in apoB and LDL-C levels were observed in the placebo group at 26 weeks ($P = .019$ and $P = .048$, respectively).

Tirzepatide 15 mg decreased LDL-C levels over time (Figure 2C). At 26 weeks, tirzepatide 15 mg and dulaglutide decreased LDL-C levels by 19.0% (−36.0%, −1.9%; $P = .029$) and 17.8% (−33.7%, −2.0%; $P = .028$) (LSM [95% CI]), respectively, compared with placebo (Figure 2D). The magnitude of reduction in LDL-C levels did not differ between tirzepatide and dulaglutide.

Tirzepatide dose-dependently decreased non-HDL-C levels over time (Figure **S1**A). At 26 weeks, tirzepatide 5 mg, 10 mg and 15 mg and dulaglutide significantly decreased non-HDL-C levels by 16.4% (−26.8%, −6.0%; $P = .002$), 21.3% (−31.9%, −10.7%; $P < .001$), 24.8% (−36.0%, −13.6%; $P < .001$) and 17.8% (−28.3%, −7.4%; $P < .001$) (LSM [95% CI]), respectively, compared with placebo (Figure **S1**B). Non-HDL-C levels increased from baseline to 26 weeks in the placebo group ($P < .001$).

3.3 | Lipoprotein particle subclasses

At 26 weeks, tirzepatide 5 mg, 10 mg and 15 mg decreased large TRLP by 59.5% (−75.6%, −32.6%; $P < .001$), 83.4% (−90.1%, −72.1%; $P < .001$) and 75.6% (−86.0%, −57.4%; $P < .001$) compared with placebo, and by 44.0% (−65.8%, −8.1%; $P = .022$), 77.0% (−86.2%, −61.9%; $P < .001$) and 66.2% (−80.3%, −41.8%; $P < .001$) (LSM [95% CI]) compared with dulaglutide, respectively (Figure 3A).

At 26 weeks, tirzepatide 10 mg and 15 mg also decreased small LDLP by 23.5% (−43.3%, −3.6%; $P = .021$) and 32.4% (−53.7%, −11.1%; $P = .003$) compared with placebo, and by 20.4% (−39.7%, −1.1%; $P = .038$) and 29.3% (−50.2%, −8.5%; $P = .006$) (LSM [95% CI]) compared with dulaglutide, respectively (Figure 3B).

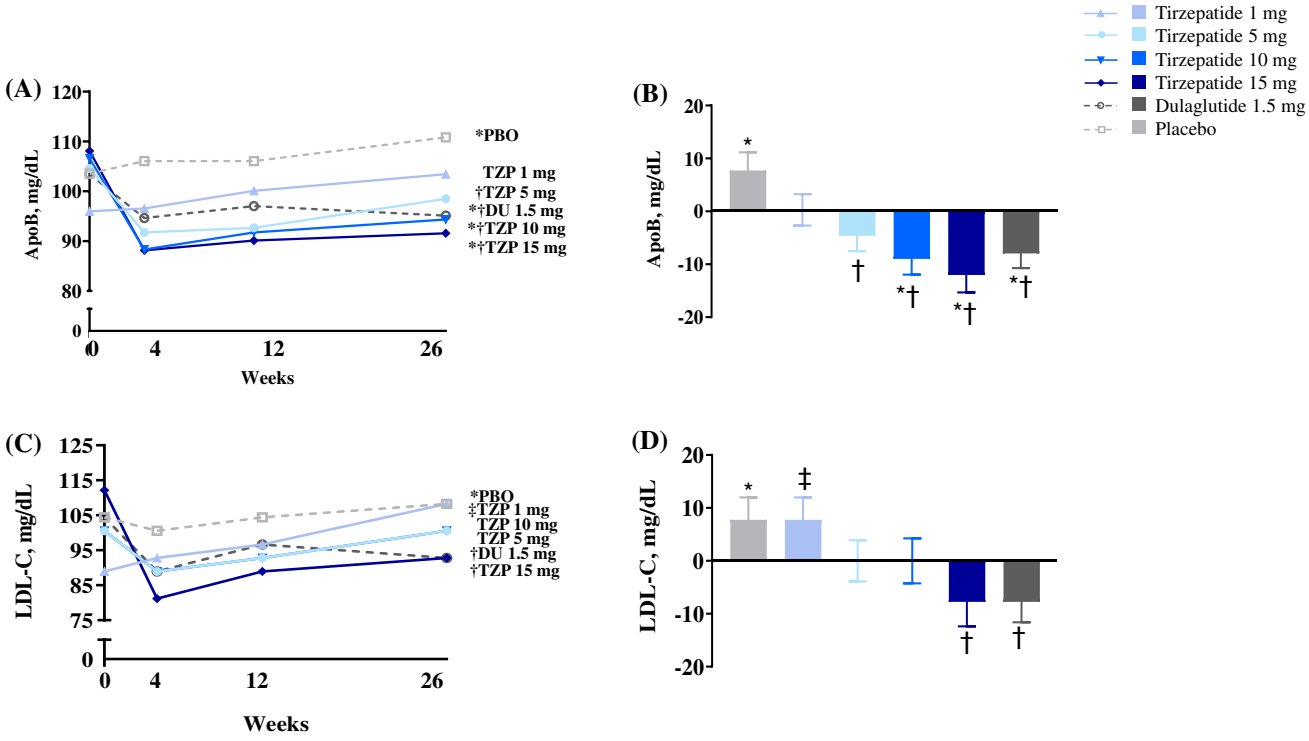


FIGURE 2 ApoB and LDL-C over time and change from baseline at 26 weeks. A, Change from baseline over time in ApoB. B, Change from baseline at 26 weeks in ApoB. C, Change from baseline over time in LDL-C. D, Change from baseline at 26 weeks in LDL-C. Data are presented as LSM actual values over time and LSM (SE) change from baseline at 26 weeks from the mITT population (placebo, n = 51; tirzepatide 1 mg, n = 52; tirzepatide 5 mg, n = 55; tirzepatide 10 mg, n = 51; tirzepatide 15 mg, n = 53; dulaglutide 1.5 mg, n = 54). ApoB is presented as antilog. *$P < .05$ vs. baseline, †$P < .05$ vs. placebo, ‡$P < .05$ vs. dulaglutide. ApoB, apolipoprotein B; DU, dulaglutide; LDL-C, low-density lipoprotein cholesterol; PBO, placebo; TZP, tirzepatide

Percent change from baseline to week 26 in all lipoprotein particle concentrations and size are presented in Table S3. At 26 weeks, tirzepatide 10 and 15 mg decreased the mean TRLP size compared with placebo and dulaglutide. Tirzepatide 5 mg, 10 mg and 15 mg decreased total LDLP concentrations compared with placebo, whereas large LDLP concentrations and mean LDLP size were increased compared with dulaglutide. Total and large LDLP concentrations increased in the placebo group. Tirzepatide 10 and 15 mg decreased the overall LPIR score by 33.5% and 31.7% compared with placebo and dulaglutide, respectively. At 26 weeks, tirzepatide 15 mg decreased total and small HDLP compared with both placebo and dulaglutide. HDL-C and apoA-I levels did not change with tirzepatide.

3.4 | Preheparin serum LPL mass

At 26 weeks, preheparin serum LPL mass dose-dependently increased from baseline with tirzepatide 5 mg, 10 mg and 15 mg by 9.4 ng/mL (2.0, 16.9; P = .013), 10.1 ng/mL (2.5, 17.7; P = .009) and 11.2 ng/mL (3.2, 19.3; P = .006) (LSM [95% CI]), respectively, compared with placebo (Figure 4).

3.5 | Lipoprotein profile in baseline triglyceride subgroups

The dose-dependent decrease in triglycerides and apoC-III with tirzepatide 5 mg, 10 mg and 15 mg was larger in patients with baseline triglyceride levels greater than or equal to 150 mg/dL compared with those with baseline triglycerides of less than 150 mg/dL. The highest dose of tirzepatide decreased triglyceride levels by 23% (Figure 5A) and apoC-III by 38% from baseline (Figure 5B) in those with triglycerides of less than 150 mg/dL compared with 43% (Figure 5C) and 47% (Figure 5D), respectively, for those with baseline triglycerides greater than or equal to 150 mg/dL. In patients with high baseline triglyceride levels (>150 mg/dL), the proportion of those reaching triglyceride levels of less than 100 mg/dL at 26 weeks was significantly higher for tirzepatide 10 mg and 15 mg compared with dulaglutide (25%, 15% and 9%, respectively; Table S4).

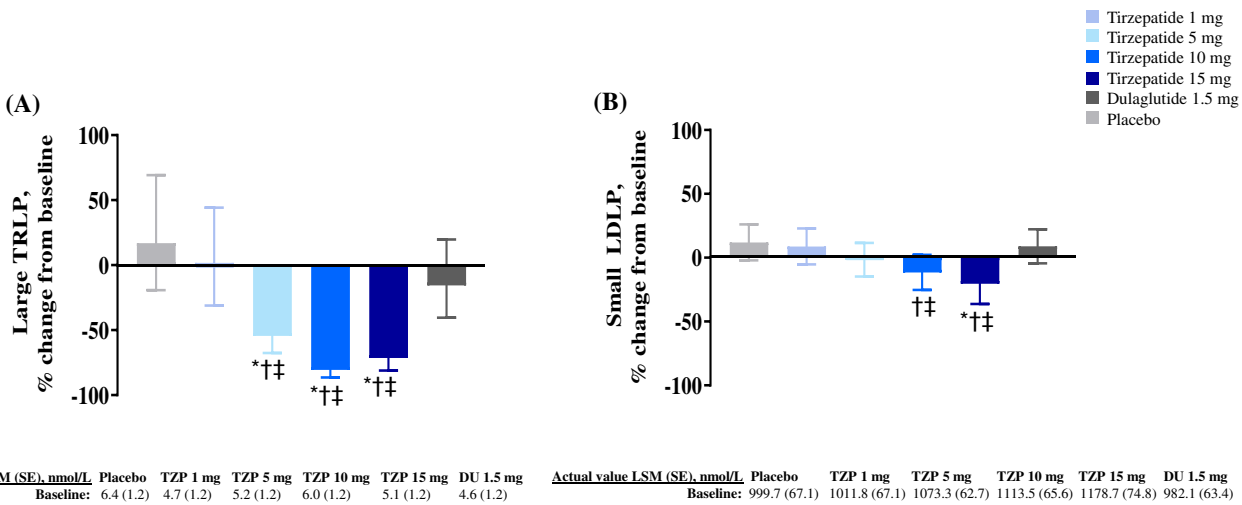
At 26 weeks, the effect of tirzepatide on LDL-C levels was similar in patients with baseline triglycerides of both less than and greater than or equal to 150 mg/dL (Figure S2A,C), whereas tirzepatide 10 mg and 15 mg resulted in a larger reduction of small LDLP in patients with baseline triglycerides greater than or equal to 150 mg/dL (Figure S2B,D).

3.6 | Predictors of triglyceride changes

Based on the variable selection procedure described in the Methods, body weight change, HbA1c change, apoC-III change, baseline body weight and sex entered the regression model explaining triglyceride changes (Table S5). Changes in apoC-III levels explained up to 22.9% of the variability in triglycerides in the two highest tirzepatide dose groups, whereas tirzepatide-induced weight loss only explained up to 4.4%.

4 | DISCUSSION

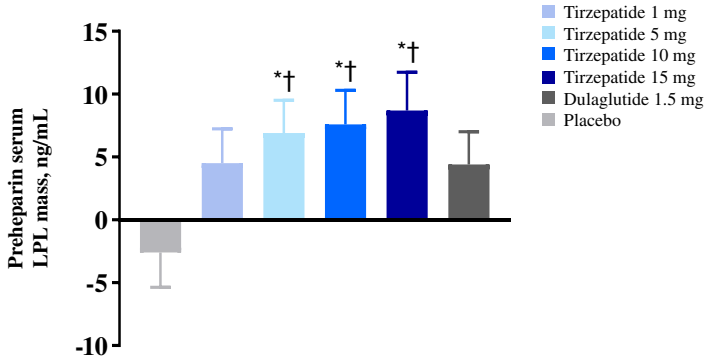
In this paper, we present the effects of the novel dual GIP and GLP-1 receptor agonist, tirzepatide, on additional lipid and lipoprotein biomarkers measured in our randomized, double-blind, controlled phase



Actual value LSM (SE), nmol/L	Placebo	TZP 1 mg	TZP 5 mg	TZP 10 mg	TZP 15 mg	DU 1.5 mg
Baseline:	6.4 (1.2)	4.7 (1.2)	5.2 (1.2)	6.0 (1.2)	5.1 (1.2)	4.6 (1.2)

Actual value LSM (SE), nmol/L	Placebo	TZP 1 mg	TZP 5 mg	TZP 10 mg	TZP 15 mg	DU 1.5 mg
Baseline:	999.7 (67.1)	1011.8 (67.1)	1073.3 (62.7)	1113.5 (65.6)	1178.7 (74.8)	982.1 (63.4)

FIGURE 3 Major lipoprotein particle subclass changes at 26 weeks. A, Percent change from baseline at 26 weeks in large TRLP. B, Percent change from baseline at 26 weeks in small LDLP. Data are presented as actual value LSM (SE) at baseline and LSM % change from baseline (95% CI) at 26 weeks from the mITT population (placebo, n = 51; tirzepatide 1 mg, n = 52; tirzepatide 5 mg, n = 55; tirzepatide 10 mg, n = 51; tirzepatide 15 mg, n = 53; dulaglutide 1.5 mg, n = 54). Large TRLP is presented as antilog. Note that large TRLP includes 'large' and 'very large' particles. *P < .05 vs. baseline, †P < .05 vs. placebo, ‡P < .05 vs. dulaglutide. DU, dulaglutide; LDLP, low-density lipoprotein particles; TRLP, triglyceride-rich lipoprotein particles; TZP, tirzepatide

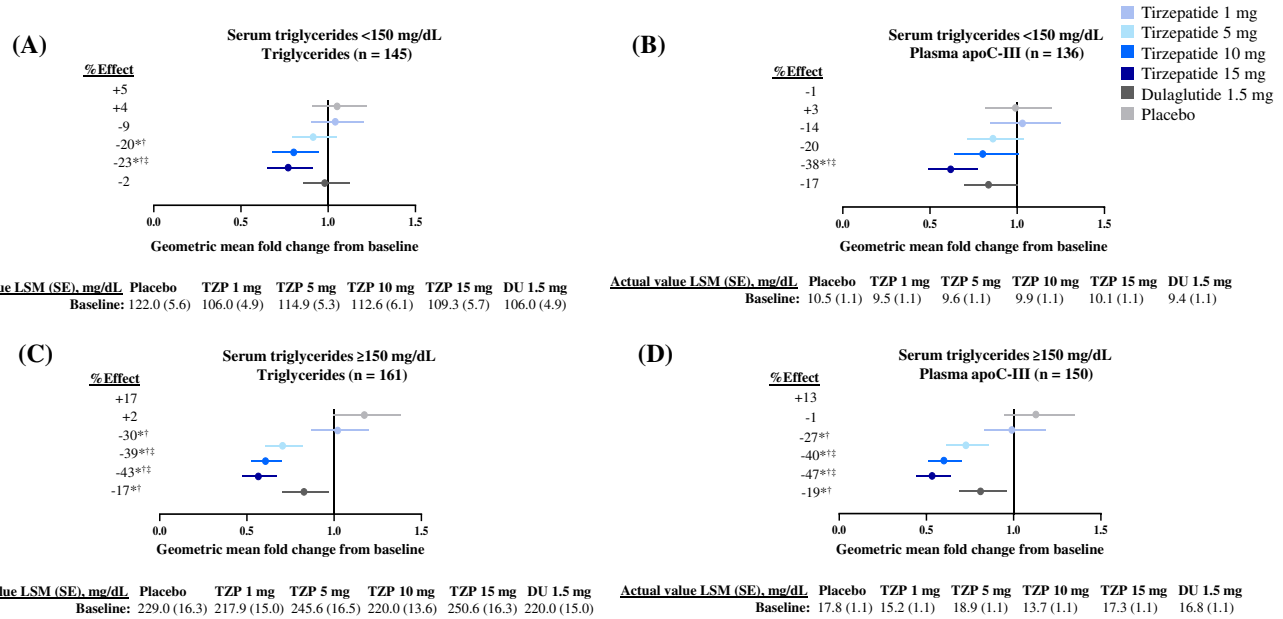


Actual value LSM (SE), ng/mL	Placebo	TZP 1 mg	TZP 5 mg	TZP 10 mg	TZP 15 mg	DU 1.5 mg
Baseline:	59.2 (3.6)	60.3 (3.7)	60.1 (3.5)	63.2 (3.6)	60.2 (3.9)	65.6 (3.6)

FIGURE 4 Change from baseline to week 26 in preheparin serum LPL mass. Data are presented as actual value LSM (SE) at baseline and LSM (SE) change from baseline at 26 weeks from the mITT population (placebo, n = 51; tirzepatide 1 mg, n = 52; tirzepatide 5 mg, n = 55; tirzepatide 10 mg, n = 51; tirzepatide 15 mg, n = 53; dulaglutide 1.5 mg, n = 54). *P < .05 vs. baseline, †P < .05 vs. placebo. DU, dulaglutide; LPL, lipoprotein lipase; TZP, tirzepatide

2b study in patients with T2D.[26] Overall, tirzepatide dose-dependently decreased triglyceride, apoB and apoC-III levels over time and decreased the number of large TRLP and small LDLP. The most robust changes were observed with the highest doses of tirzepatide (10 and 15 mg).

Serum triglycerides had an average reduction of 31% and 35% and apoC-III had an average reduction of 32% and 44% from baseline with the two highest doses of tirzepatide (10 and 15 mg, respectively). These changes are remarkable compared with those reported for effective triglyceride-lowering drugs, like fenofibrate, in patients with T2D (triglycerides were reduced by 26% and apoC-III by 20%).[7,29] Up to 25% of the patients with high baseline triglyceride levels treated with tirzepatide reached triglyceride levels of less than 100 mg/dL at 26 weeks. Although apoB levels also decreased dose-dependently, the percent change from baseline was ~11% compared with 35% for triglycerides at the highest dose of tirzepatide. This finding implies that the increase in triglyceride lipolysis per particle is greater than the decrease in the number of apoB-containing particles, as suggested by the presence of a significant correlation between triglyceride and apoB levels at baseline (r = 0.29, P = .01), but not between changes in triglyceride and apoB levels at 26 weeks (r = 0.24, P = .04). The discordant changes between triglyceride and apoB levels are further supported by the marked reduction in the numbers of large TRLP and the average size of TRLP at the two highest doses of tirzepatide. Therefore, tirzepatide treatment results in smaller-sized VLDL particles that are metabolized to LDLP of medium size instead of



Actual value LSM (SE), mg/dL	Placebo	TZP 1 mg	TZP 5 mg	TZP 10 mg	TZP 15 mg	DU 1.5 mg
Baseline:	122.0 (5.6)	106.0 (4.9)	114.9 (5.3)	112.6 (6.1)	109.3 (5.7)	106.0 (4.9)

Actual value LSM (SE), mg/dL	Placebo	TZP 1 mg	TZP 5 mg	TZP 10 mg	TZP 15 mg	DU 1.5 mg
Baseline:	10.5 (1.1)	9.5 (1.1)	9.6 (1.1)	9.9 (1.1)	10.1 (1.1)	9.4 (1.1)

Actual value LSM (SE), mg/dL	Placebo	TZP 1 mg	TZP 5 mg	TZP 10 mg	TZP 15 mg	DU 1.5 mg
Baseline:	229.0 (16.3)	217.9 (15.0)	245.6 (16.5)	220.0 (13.6)	250.6 (16.3)	220.0 (15.0)

Actual value LSM (SE), mg/dL	Placebo	TZP 1 mg	TZP 5 mg	TZP 10 mg	TZP 15 mg	DU 1.5 mg
Baseline:	17.8 (1.1)	15.2 (1.1)	18.9 (1.1)	13.7 (1.1)	17.3 (1.1)	16.8 (1.1)

FIGURE 5 Change from baseline to week 26 in triglycerides and ApoC-III in patients with A and B, baseline triglycerides <150 mg/dL, respectively, and with C and D, baseline triglycerides ≥150 mg/dL, respectively. Data are presented as LSM fold change vs. baseline from the mITT population (placebo, n = 51; tirzepatide 1 mg, n = 52; tirzepatide 5 mg, n = 55; tirzepatide 10 mg, n = 51; tirzepatide 15 mg, n = 53; dulaglutide 1.5 mg, n = 54). *P < .05 vs. baseline, †P < .05 vs. placebo, ‡P < .05 vs. dulaglutide. apoC-III, apolipoprotein C-III; DU, dulaglutide; TZP, tirzepatide

generating small dense LDL,[30] thus shifting the LDL distribution towards less atherogenic LDLP. Although postheparin hepatic lipase (HL) activity was not measured in this study, GIP receptor-specific antagonism has been shown to suppress HL activity in mice in vivo,[31] whereas postprandial GIP response has been suggested to increase HL activity in vivo in humans.[32] HL hydrolyses triglycerides and phospholipids in apoB-containing lipoproteins and HDL, resulting in the formation of small LDLP and HDLP. Indeed, HL-deficient subjects have large buoyant LDLP and large-size HDLP.[33] Treatment with tirzepatide reduced both small LDLP and HDLP compared with dulaglutide, despite no differences being observed in either the LDL-C or HDL-C levels between the two treatments. These changes in lipoprotein subclass distribution may be secondary to improved insulin sensitivity with tirzepatide.[34] Taken together, the significant reductions in small LDLP, non-HDL-C and apoB levels, in addition to reductions of triglycerides and apoC-III, point towards an overall less atherogenic lipoprotein profile in patients with T2D treated with tirzepatide.

In this study, we also observed a dose-dependent increase in preheparin plasma LPL mass during tirzepatide therapy compared with placebo, in line with previous data, demonstrating that GIP induces LPL activity in vitro and in vivo.[22,23] Notably, GIP enhances the vasodilation and capillary recruitment, resulting in an increased uptake of fatty acids from circulating triglycerides. This finding supports the increased lipolytic capacity and reduction of triglyceride levels observed with tirzepatide. However, we found no correlation between changes in preheparin LPL mass and changes in plasma triglyceride levels ($r = -0.10$, $P = .38$). This may be attributed to two causes. First, preheparin LPL in circulation only represents a trivial amount of the actual enzyme at endothelial surfaces. Second, even heparin-releasable LPL activity may not completely reflect the true activity of LPL at its actual sites of action. The lack of correlation between changes in preheparin LPL mass and changes in triglycerides could also suggest a role of other determinants of triglyceride concentrations, such as apoC-III. Indeed, triglyceride clearance rates in both the fasting and postprandial states have been shown to be closely linked to plasma apoC-III levels, but not related to postheparin plasma LPL activity.[35,36] The reduction of apoC-III levels with tirzepatide is unlikely to be explained by direct GIP receptor agonism in hepatocytes because GIP receptors do not appear to be expressed in the liver.[37,38] A direct effect of tirzepatide on intestinal GIP receptors could theoretically reduce intestinal apoC-III production, but this also seems unlikely to explain reduced fasting apoC-III levels. Indirect mechanisms of tirzepatide on GIP receptors may be partially responsible for the effect of tirzepatide on apoC-III levels. As this is a descriptive study, we cannot exclude the potential contribution of a reduced large VLDL production rate to large VLDL and decreased triglycerides. A kinetic study using stable isotopes is warranted to elucidate the role of tirzepatide in the metabolism of fasting and postprandial TRLs in humans.

In the current study, the magnitude of reduction of triglyceride and apoC-III levels was very similar at all efficacious doses of tirzepatide in patients with either normal (<150 mg/dL) or elevated baseline triglycerides. We also observed strong correlations between changes in apoC-III and changes in triglycerides for all dose groups, with an r value of 0.69 for the two combined highest doses. Finally, changes in apoC-III levels were the strongest predictor of changes in serum triglycerides with tirzepatide, explaining ~23% of the variance in a regression model that also included changes in HbA1c and body weight among other variables. This is not unexpected, as apoC-III is critical for triglyceride metabolism, through inhibition of LPL activity and impairment of triglyceride-rich lipoprotein clearance by promoting the uptake of TFL remnants by the liver.[8,36] Weight loss with bariatric surgery is consistently accompanied not only by HbA1c reduction, but has also resulted in markedly reduced plasma triglycerides and apoC-III, and this decrease was accompanied by a redistribution of apoC-III from triglyceride-rich lipoproteins to HDL.[39] However, although tirzepatide resulted in better glucose control and greater weight loss than dulaglutide, the multiple linear regression analysis indicated that tirzepatide-induced weight loss only explained a small portion of the variability in triglycerides.

This raises the issue of why apoC-III was reduced by tirzepatide in these subjects with diabetes. It is well known that the expression of apoC-III is regulated by both plasma glucose and insulin, but in opposite directions.[7] In fact, the transcription rate of apoC-III has been reported to be inhibited by insulin via PPAR-α and FXR,[8,40,41] whereas glucose has been shown to stimulate expression of apoC-III via HNF-4α and ChREBP.[42] However, even though insulin resistance and T2D hyperglycaemia and hyperinsulinemia co-exist, VLDL overproduction and hypertriglyceridaemia result from both hyperglycaemia and the inability of insulin to suppress apoC-III expression secondary to the insensitivity of FoxO1 signalling.[43–45] Plasma apoC-III concentrations are known to be increased in subjects with T2D and in those with insulin resistance,[46] and recently it was reported that apoC-III metabolism is disturbed in those subjects.[47] The apoC-III secretion rate measured using the stable isotope leucine was markedly higher in subjects with T2D than in body mass index-matched non-diabetic subjects. Treatment with liraglutide (1.8 mg/day) for 16 weeks reduced the apoC-III concentration by 14%, and changes in plasma apoC-III were significantly associated with changes in HbA1c, supporting the role of glucose homeostasis as a regulator of apoC-III levels and apoC-III production.[47] In the current study, changes in apoC-III levels were also significantly associated with changes in HbA1c at the two highest doses ($r = 0.33$, $P = .005$), but not with changes in HOMA-IR ($r = 0.17$, $P = .19$).

This study had limitations. First, because this was a post hoc analysis of additional biomarkers measured in stored samples, the sample size calculated for the original clinical trial could be inappropriate for these analyses. However, results were similar when the two highest dose groups of tirzepatide were analysed as combined, considering that they had similar efficacy. Second, all lipids, lipoproteins and apolipoproteins were measured on a single fasting plasma sample at different times of the 26-week study. However, static measurements in the fasting state remain descriptive and do not reveal underlying mechanisms, therefore, stable isotope kinetic studies in the postprandial state are needed to better understand the mechanistic dynamics of the lipid changes during tirzepatide therapy.

In conclusion, the dose-dependent decrease in apoC-III levels appears to partially explain the dose-dependent reduction in triglycerides following tirzepatide treatment, independently of weight loss. Moreover, the reduction in the number of large TRLP and small LDLP and apoB levels is consistent with a net improvement in insulin sensitivity and atherogenic lipoprotein profile following tirzepatide treatment. The planned cardiovascular outcomes study SURPASS-CVOT (NCT04255433) should provide definitive data on the potential further cardiovascular benefit of the dual GIP and GLP-1 receptor agonist, tirzepatide, above and beyond treatment with a GLP-1 receptor agonist alone.

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CONFLICT OF INTERESTS

JMW, AN, WCR, DAR, JSR, AH, KLD and GR are employees and shareholders of Eli Lilly and Company. M-RT reports grants and personal fees from Amgen, NovoNordisk, and personal fees from Sanofi and Akcea.

AUTHOR CONTRIBUTIONS

JMW, AN, DAR, AH, KLD and GR contributed to the study design. AH and GR provided medical oversight during the trial. AN was responsible for the statistical analyses. GR and AH are the guarantors of this work and, as such, take responsibility for the integrity of the data and the accuracy of the data analysis. All authors participated in interpretation of the data and critical review of the manuscript, had full access to all the data in the study and approved this manuscript to be submitted for publication.

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SUPPORTING INFORMATION

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